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Flow characterization and patch clamp dose responses using jet microfluidics in a tubeless microfluidic device

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HIGHLIGHTS

- We perform patch-clamp studies inside a tubeless microfluidic device using surface tension passive pumping and derivatives.
- We compare TIRF imaging and electrical recordings for measuring fluid exchanges in microfluidic channels for patch clamping.
- The novel method works well to obtain peak dose responses but is not suitable to study the kinetics of most ion channels.

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ABSTRACT

Background: Surface tension passive pumping is a way to actuate flow without the need for pumps, tubing or valves by using the pressure inside small drop to move liquid via a microfluidic channel. These types of tubeless devices have typically been used in cell biology. Herein we present the use of tubeless devices as a fluid exchange platform for patch clamp electrophysiology.
New method: Inertia from high-speed droplets and jets is used to create flow and perform on-the-fly mixing of solutions. These are then flowed over GABA transfected HEK cells under patch in order to perform a dose response analysis.
Results: TIRF imaging and electrical recordings are used to study the fluid exchange properties of the microfluidic device, resulting in 0–90% fluid exchange times of hundreds of milliseconds. COMSOL is used to model flow and fluid exchange within the device. Patch-clamping experiments show the ability to use high-speed passive pumping and its derivatives for studying peak dose responses, but not for studying ion channel kinetics.
Comparison with existing method(s): Our system results in fluid exchange times slower than when using a standard 12-barrel application system and is not as stable as traditional methods, but it offers a new platform with added functionality.
Conclusions: Surface tension passive pumping and tubeless devices can be used in a limited fashion for electrophysiology. Users may obtain peak dose responses but the system, in its current form, is not capable of fluid exchange fast enough to study the kinetics of most ion channels.

1. Introduction

The term “jet microfluidics” has been used to refer to ink jet printing (Lee, 1974), the injection of medication through the skin (Lambert and Laurent, 2008), or in multiphase emulsion systems (Sauret et al., 2012; Guillot et al., 2009). The term has also been used to describe jets impinging on hydrophobic surfaces (Celestini

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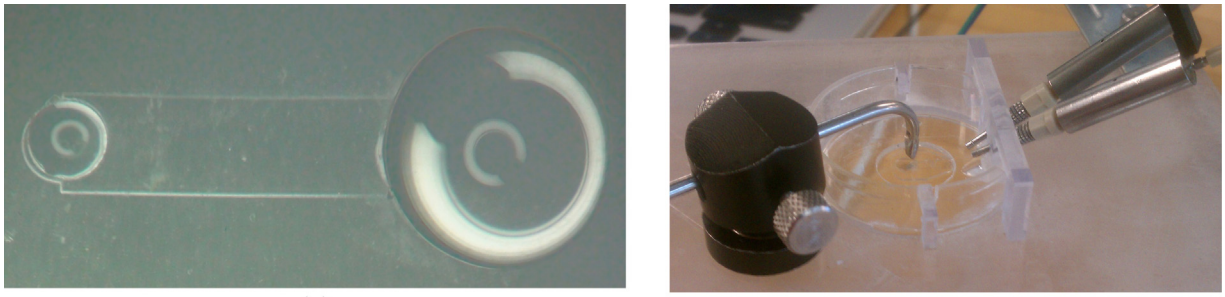

Fig. 1. (a) PDMS microfluidic device. (b) PDMS device (transparent) placed on a plastic substrate, with nozzles aimed atinlet and vacuum tube at outlet.

et al., 2010). Here we define jet microfluidics as the use of liquid jets to create flow within a tubeless microfluidic device by using the dynamic pressure of a fluid droplet, or jet, impinging on a liquid surface (Resto et al., 2012). A tubeless microfluidic device is one that does not require a tube to deliver liquid to the microfluidic device, such as the one used in this paper. Other types of tubeless microfluidic devices have mostly been used with passive flow mechanisms such as surface tension (Walker and Beebe, 2002), evaporation (Lynn et al., 2009; Scott Lynn and Dandy, 2009), and capillary action (Stone et al., 2004) or with electro-osmotic flow on an open surface (Barbulovic-Nad et al., 2010). These types of systems have mostly been used for cell biology assays (Barbulovic-Nad et al., 2010; Meyvantsson et al., 2008; Khnouf et al., 2009; Du et al., 2009). We have previously developed a set of techniques called "inertia enhanced passive pumping" and "inertia actuated flow" where the inertia of high-speed droplets ejected from micronozzles is used to create flow within a tubeless microfluidic device (Resto et al., 2012). These techniques evolved from lessons learned by studying surface tension passive pumping (Walker and Beebe, 2002). In this context, jet microfluidics allow the use of tubeless microfluidic devices for applications where active control over flow is desired, with steady flow rates and rapid fluid exchanges as is required, for example, in electrophysiology. Without the inertia component in flow actuation, liquid flow would be dominated by surface tension, and would more aptly be named surface tension passive pumping (Walker and Beebe, 2002). Under surface tension passive pumping alone, the flow rates are too low and the exchange times are too great for use in electrophysiology.

Ion channels are pore-forming proteins that extend across cell membranes and selectively allow ions to pass into and out of cells (Hille, 2001). They are important drug targets, as they play a key role in many physiological processes (Kaczorowski et al., 2008). High throughput screening techniques and automated electrophysiology tools are being developed to identify new compounds that can activate or modulate ion channels (Sigworth and Klemic, 2005; Bao et al., 2008; Fertig et al., 2002). A number of different commercial platforms are available for high throughput studies of voltage-activated ion channels (Priest et al., 2007). However, because of the challenges inherent in exchanging the solutions around cells while maintaining stable electrophysiological recordings, only a handful of such systems have been developed for studying ligand-gated ion channels. The typical goal of such studies is to measure the activation, desensitization and deactivation of ligand-gated ion channels, which operate under non-steady state conditions, on the order of milliseconds to hundreds of milliseconds (Farrant and Nusser, 2005). The usual approach to achieving fluid exchange around a membrane patch or cell expressing receptors of interest has been through the use of the 'liquid filament' technique, wherein side-by-side liquid streams are physically translocated (Franke et al., 1987).

To conduct electrophysiology experiments, setups are typically composed of multiple channels in parallel, each delivering ligands, agonists, antagonists, different drugs or drug concentrations to a patched pipette or a planar pore. For multiple drugs or drug concentrations, this would mean one tube per variation. A library of hundreds of drugs can make for a quite bulky setup. Surface tension passive pumping (Resto et al., 2012; Walker and Beebe, 2002; Resto et al., 2010) provides the potential to screen a library of drugs using an open microfluidic device. In this setup, the liquid delivery unit is separate from the channel without any attached tubing at the inlet or the outler (Walker and Beebe, 2002). The pressure inside the microfluidic channel is not greater than ambient, since it is open to ambient at inlet and outlet. Therefore a center slit can be cut halfway through the channel, offering the ability to insert recording equipment into the microfluidic channel while a cell is patched and flow is occurring. This offers the potential to perform simultaneous recording experiments, with characterized flow and fluid exchange properties, where liquid is compartmentalized yet is accessible through various entry points. This type of tubeless device can be used in an automated liquid handler to perform high-throughput screening experiments.

The current work characterizes the use of jet microfluidics in a tubeless device for patch clamping electrophysiology by studying the flow properties inside the microfluidic device. It then applies jet microfluidics and tubeless devices to patch-clamping electrophysiology. Fluid velocities are measured using Particle Image Velocimetry (PIV) and fluid exchange times are measured using electrical recordings across an open micro-pore and total internal reflection fluorescence (TIRF) microscopy. These results are then compared to a COMSOL (COMSOL Inc, Burlington, MA) computer simulation in order to validate the physical phenomena dominating fluid exchanges. We report of the use of jet microfluidics in a tubeless microfluidic device as a fluid application system for patch clamp recording, taking advantage of open surfaces with confined flow. The system reduces the number of reservoirs used in a dose response protocol while not requiring any moving parts from inside the device.

2. Methods

2.1. Microfluidic system

Microfluidic devices are made out of PDMS using conventional soft lithography methods (Duffy et al., 1998). The microfluidic devices used throughout all experiments consist of a straight rectangular channel (dimensions 280 μm height, 2 mm width and 1 cm length) with circular inlet and outlet ports (radii 1 mm and 2 mm, respectively), as shown in Fig. 1(a). The inlet drop radius is made to be equal to or less than the inlet port radius to minimize liquid spillage. The PDMS device is placed on top of a substrate, glass or plastic according to the experiment, where it is aimed at the